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# **An interproximal model to determine the erosion-protective effect of calcium silicate, sodium phosphate, fluoride formulations**

Natalie J Wood<sup>a</sup>, Siân Bodfel Jones<sup>a</sup>, Nick Chapman<sup>a</sup>, Andrew Joiner<sup>b</sup>, Carole J Philpotts<sup>b</sup>, Nicola X West<sup>a</sup>

<sup>a</sup>Clinical Trials Group, School of Oral and Dental Sciences, University of Bristol, Lower Maudlin Street, Bristol, BS1 2LY, UK

<sup>b</sup>Unilever Oral Care, Quarry Road East, Bebington, Wirral, CH63 3JW, UK

## **Author email addresses:**

NJ Wood, natjanewood@gmail.com; SB Jones, s.b.jones@bristol.ac.uk; N Chapman, nick.chapman@bristol.ac.uk; A Joiner, andrew.joiner@unilever.com; CJ Philpotts, carole.philpotts@unilever.com; NX West, n.x.west@bristol.ac.uk.

**Short title:** Calcium silicate, sodium phosphate formulations protective effect against erosive challenge.

## **Corresponding Author**

Professor Nicola West,  
Periodontology Clinical Trials Unit,  
Bristol Dental School,  
Lower Maudlin Street,  
Bristol, BS1 2LY, UK.  
Tel: +44 (0)117 342 5903;  
fax: +44 (0)117 342 4000.  
*E-mail address:* n.x.west@bristol.ac.uk (N.X. West).

## **Conflicts of interest and funding statement**

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# **An interproximal model to determine the erosion-protective effect of calcium silicate, sodium phosphate, fluoride formulations**

## **Abstract**

**Objectives:** Previous work has shown the effectiveness of a newly developed interproximal model to differentiate between the amount of remineralization caused by toothpastes used with or without a dual-phase gel treatment system containing calcium silicate, sodium phosphate salts and fluoride to repair acid-softened enamel. The aim of this study was to utilize the same interproximal model to identify how effective calcium silicate phosphate toothpastes are at reducing surface softening in the early stages of erosion. The model was also used to identify the effect of increasing the frequency of acid exposure on the reduction in surface hardness.

**Methods:** Human enamel specimens were prepared and mounted in an interproximal face-to-face arrangement and exposed to a cycling regime of whole human saliva, treatment, artificial saliva and 1 % citric acid pH 3.75. Specimens were measured by surface microhardness at baseline and after three and seven days. The frequency of acid exposure was increased from 2 to 4 cycles a day for the second part of the study.

**Results:** The results showed that specimens treated with the calcium silicate phosphate toothpastes softened less than those treated with control fluoridated or non-fluoride toothpastes at each time point and following an increase in the frequency of acid exposure.

**Significance:** This work has demonstrated how an interproximal model can also be successfully used to determine differences in the erosion protection of various treatments as well as determining how they perform when the frequency of acid exposure is increased.

**Key Words:** erosion, Calcium phosphate sodium silicate, Enamel, Interproximal model, toothpaste

## 1.0 Introduction

Within the oral cavity dental hard tissues are exposed to many factors that could lead to wear and mineral loss [1]. Dietary acids, such as those found in soft drinks, are a very common cause of enamel erosion within the mouth; the most prevalent of which is citric acid [2].

The investigation of dental erosion has increased dramatically over the last fifteen years and for reasons of reproducibility and ease of measuring most of the *in vitro* work has been carried out on flat, polished enamel surfaces in an open environment with a free flow of solution around the enamel specimen [3]. It could be argued that this does not fully represent the environment in the oral cavity as within the oral cavity there are several crevices and interproximal areas where the fluid flow around the region is not as freely flowing as in an open environment. The enamel found at the interproximal region of the tooth is exposed to a unique environment, when compared to the buccal/labial or the lingual/palatal surfaces, due to the potential confinement of bacteria and/or acidic food and drinks next to the tooth surface within the interdental space [4, 5]. The space below the interdental point varies from tooth to tooth and can be as much as 6 mm in length down to the cemento enamel junction [6] where the enamel thins into nothing thus making it more susceptible to enamel loss and subsequent pain caused by sensitivity [7]. Until recently, the only *in vitro* interproximal models were designed to simulate caries; however, it is also clinically relevant to determine the effect of erosive acid exposure on these potentially more vulnerable regions, especially as little is known about this. An *in vitro* interproximal erosion model has previously been described in the literature and been used to successfully demonstrate the remineralization effect of certain toothpastes on previously demineralized enamel, when mounted interproximally [8]. The model consisted of individual bovine enamel pieces mounted so that exposed enamel surfaces were set face-to-face with an approximate 100µm space between both pieces of enamel to simulate the interproximal space and allow local reactions to take place that might also be taking place in the oral environment. This previous study highlighted a difference in the rate of remineralization observed when specimens in an open environment were treated with the same toothpastes as those using the interproximal model [9]. The magnitude of remineralization measured using the open environment model was greater than that observed using the interproximal model, thus highlighting the importance of further investigating the rate of erosion of specimens using the interproximal model.

It is known that the presence of calcium and phosphate in oral care products can increase the concentration of those ions in the saliva. These increased concentrations mean the saliva has a higher degree of saturation with respect to calcium and phosphate ions, reducing the rate of enamel dissolution induced by a low oral pH from the presence of extrinsic and/or intrinsic acids [10]. There have been many developments in toothpaste manufacturing to include agents that are effective in reducing dental erosion specifically, as

this is recognized as requiring a different approach to that of dental caries [11-13]. A recently developed oral care product combines the use of calcium silicate, sodium phosphate salts and fluoride in a novel treatment system to help protect sound enamel from erosive attacks, while repairing acid-softened enamel [8, 9, 14, 15]. The system involves the use of a calcium silicate and sodium phosphate salts plus fluoride containing toothpaste, in conjunction with a dual-phase gel used for 3 consecutive days, once a month. Enamel is protected and repaired through the deposition of calcium silicate which facilitates the nucleation of hydroxyapatite (HAP), the predominant mineral in teeth [14, 16]. Tooth mineral loss from acid challenges has been shown to be significantly decreased by the application of calcium silicate and by the combined use of calcium silicate and fluoride [8, 9, 13].

The aim of this work was to employ the previously developed interproximal model to determine the effectiveness of calcium silicate, sodium phosphate salts and fluoride containing oral care products in reducing surface softening of human enamel *in vitro* and to identify the effect of increasing the frequency of acid exposure. The null hypotheses tested were 1) there are no differences in the amount of surface softening that takes place following treatment with each of the products tested at each time point and 2) an increase in the frequency of acid exposure does not influence the amount of surface softening of the enamel following treatment.

## **2.0 Materials and Methods**

### ***2.1 Preparation of enamel specimens***

Human molars sourced from a HTA licensed tissue bank (REC 11/NI/0145) were sectioned under irrigation into enamel specimens using a microslice (Ultratec, Santa Ana, CA, USA). The flattest surface of the sectioned enamel was briefly ground on a rotating polishing machine using a silicon carbide disc (p1200), to form a flat enamel face. These specimens were then embedded face down and slightly off center in resin (Stycast 1266, Hitek Electronic Material Ltd, South Humberside, UK). The blocks produced were subsequently ground flat using a silicon carbide disc (p1200), followed by polishing in a slurry of silica powder (p1200) (Kemet, Kent, UK) in deionized (DI) water on a glass slab, by hand, until the enamel surface was level and fully exposed. The samples were rinsed and then sonicated in DI water for 10 minutes, before being polished to a flat shine using a slurry of 0.3  $\mu\text{m}$  alumina powder (Kemet, Kent, UK) and DI water, on a felt pad (Kemet, Kent, UK). The samples were again rinsed and sonicated in DI water for 10 minutes.

Baseline microhardness measurements were taken for all specimens; the specimens were then grouped such that each treatment group had a similar range of hardness values. Several specimens from each group were also subjected to SEM imaging, prior to

experimentation. Following baseline measurements, the specimens were paired and attached together using double-sided tape so that the enamel faces were set face-to-face at an approximate distance of 100  $\mu\text{m}$  apart. Pairs of blank resin blocks, i.e. containing no enamel specimens, were placed on both ends to ensure uniform exposure of the test specimens. Each group consisted of 8 specimen pairs (16 total specimens), and was subjected to a unique treatment regime.

## *2.2 Sample measurement*

Prior to treatment, each specimen was indented 6 times using a Vickers diamond tip on a Duramin 1 indenter (Struers, Rotherham, UK). Each specimen was then indented 6 times at each measuring time point. A force of 1.96 N was applied for 20 s. Indent measurements were taken under a magnification of  $\times 40$ .

Representative specimens from each group were imaged at baseline, and after days 3 and 7, using a Phenom G2 pro desktop SEM (Phenom World, Eindhoven, The Netherlands).

## *2.3 Study one: Protection of interproximal spaces with low frequency acid exposure*

On day 1, all specimens were incubated in whole, pooled human saliva at 37°C for 1 h. Saliva was sourced through an HTA licensed NHS Research Ethics Committee approved saliva bank (REC 08/H0606/87+5). Specimens were then removed, rinsed with DI water and exposed to their assigned treatment. Toothpaste slurries were prepared through rapid homogenization of toothpaste in DI water (1:2), using an IKA T25 Digital Ultra Turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany). Treatments were as highlighted in Table 1.

The slurries were applied to the top of the specimen pairs during 10 s, before being allowed to flow between the specimens for 1 min as previously described [8] and highlighted in Figure 1. In the case of the toothpaste + dual-phase gel treatment group, after the pairs had been rinsed with DI water, the gel was applied to the top of pairs and left for 3 min. The dual phase gel was used as supplied. The specimen pairs were rinsed in DI water for a final time, and placed in artificial saliva, before all specimen groups were incubated overnight at 37°C.

At the start of each subsequent treatment day, the specimen pairs were removed from the artificial saliva, rinsed in DI water and incubated at 37°C in whole, pooled human saliva for 1h. Specimen pairs were removed, rinsed in DI water and exposed to the relevant treatment. The morning treatment for specimens in the paste + dual phase gel group consisted only of the toothpaste, and no gel. After treatment, all specimen pairs were rinsed in DI water before being immersed in fresh artificial saliva for 2 h. All samples were then removed, rinsed in DI water and immersed in 1% citric acid (pH 3.75) at room temperature

(~20°C) for 2 min. After which, specimen pairs were rinsed in DI water and placed back in artificial saliva; the artificial saliva/citric acid cycle was repeated once more. Specimen pairs were then rinsed, and immersed in pooled, whole human saliva for 1 h at 37°C, which was followed by a treatment step described as for day 1. For the paste + dual phase gel treatment group, the afternoon treatments included application of the gel for days 1, 2 and 3, after which (days 4-7) only the paste was administered. An overview of the cycling procedure is given in Figure 2.

After days 3 and 7, specimens were incubated at 37°C overnight in artificial saliva; the following day all specimens were un-taped and thoroughly rinsed with DI water. The microhardness of all specimens was then determined, along with imaging of some specimen surfaces from each treatment group under SEM.

#### *2.4 Study two: Protection of interproximal spaces with high frequency acid exposure*

Study two was carried out as study one, apart from each day contained 4 acid exposures of 2 min, separated by a 1 h soak in artificial saliva, as opposed to 2 exposures of 2 min, separated by a 2 h soak in artificial saliva described for study one. All other times were consistent, as were assigned treatments.

#### *2.5 Statistical analysis*

All treatment groups within each study were compared at each time point using a one-way analysis of variance (ANOVA), followed by a Tukey post-hoc test. The difference from baseline values for both studies were also compared to determine the effect that increasing the acid frequency had on the amount of surface softening. For this comparison an independent sample t test was carried out at each time point. A p-value of 0.05 was chosen to indicate significance and calculations were carried out using SPSS version 23 (IBM, New York, USA).

### **3.0 Results**

#### *3.1 Study 1*

The hardness values of all specimens at baseline were similar at approximately 350 VHN (Table 2). After 3 and 7 days of treatment the amount of surface softening was greatest for the specimens in the non-fluoride toothpaste group and lowest for the specimens in Groups 1 and 2 that had been treated with either the calcium silicate sodium phosphate toothpaste slurry with and without the dual-phase gel system. After 7 days of treatment, there was no significant difference in the reduction in surface microhardness of the calcium silicate and

sodium phosphate toothpaste slurry with and without the dual-phase gel system but these the microhardness of these groups was significantly less than for the control groups that were treated with either the SMFP-containing toothpaste or the non-fluoridated toothpaste (Figure 3a).

After 3 and 7 days of treatment the specimens in groups 3 and 4, treated with either the SMFP toothpaste or the non-fluoridated toothpaste showed a significant reduction in surface hardness from baseline with the non-fluoridated toothpaste also showing a significant increase in surface softening from day 3 to day 7. The amount of surface softening following treatment with the calcium silicate sodium phosphate toothpaste both with and without the dual-phase gel did not show a significant difference from baseline at any time point.

SEM images taken following baseline, day three and day seven treatments showed a greater amount of erosion as evidenced by a more pronounced fish scale pattern for groups 3 and 4 that had been treated with the SMFP containing toothpaste and the non-fluoridated toothpaste, than for groups 1 and 2, treated with calcium silicate sodium phosphate toothpaste with or without the dual-phase gel. Representative images of specimens from control and test groups are shown in Figure 4.

### *3.2 Study 2*

For the second part of the study all baseline readings were again very similar with no significant difference in the baseline readings between each group (Table 3). After three days of treatment the group that showed the least amount of surface softening was the group of specimens that had been treated with the calcium silicate and sodium phosphate paste combined with the dual-phase gel. The reduction in hardness from this group was similar to the group that had been treated with the calcium silicate and sodium phosphate toothpaste alone but significantly less than the other two control groups. After 7 days of treatment the group that had been treated with the calcium silicate and sodium phosphate paste combined with the dual-phase gel again showed the least amount of reduction in hardness which again was similar to the group that had been treated with the calcium silicate and sodium phosphate toothpaste alone. The reduction in hardness was significantly different from baseline following treatment for all groups with the exception of the group that was treated with the calcium silicate and sodium phosphate toothpaste combined with the dual-phase gel which was still statistically similar to baseline following three days of treatment. This latter group also gave the overall lowest reduction in hardness from baseline after 7 days (Figure 3b).

Increasing the acid frequency from study one showed a significantly greater degree of surface softening for all the treatment groups with the exception of the group that had been



treated with the calcium silicate and sodium phosphate toothpaste combined with the dual-phase gel for three days ( $p=0.841$ ) (Table 4).

The SEM images taken for this second study showed a much greater amount of surface change compared to after treatment in the first study. Similar to the first study, following treatment, deterioration in the control groups appeared to be somewhat greater than that observed in the test groups. Example images are shown in Figure 5. In general, the amount of surface change after 7 days of treatment appeared to be much greater than after only 3 days of treatment.

#### 4.0 Discussion

The results from both studies show that the first null hypothesis stating that there are no differences in the amount of surface softening that takes place following treatment with each of the products tested at each time point can be rejected because in both studies there were significant differences between groups after three and seven days of treatment. At each time point within both studies, the amount of surface softening was significantly less for the specimens that had been treated with the calcium silicate sodium phosphate toothpaste and dual-phase gel or the calcium silicate sodium phosphate toothpaste alone compared to the groups that had been treated with either the SMFP containing toothpaste or the non-fluoride toothpaste.

The second null hypothesis which states that an increase in the frequency of acid exposure does not influence the amount of surface softening of the enamel following treatment can also be rejected with the exception of the results obtained from specimens treated with the calcium silicate and sodium phosphate toothpaste and dual-phase gel combined following three days of treatment. There was no significant difference in the amount of softening after increasing the frequency of acid exposure for this group.

There were no significant differences between the two control groups at any time point in either study suggesting that the addition of fluoride as SMFP had limited effect on reducing the amount of surface softening in this model. One explanation for this observation is that phosphatase enzymes are present in human but not artificial saliva and these are required to release the fluoride ion from SMFP [17]. Indeed, some *in vitro* studies have been described using phosphatase enzyme incubation of the SMFP containing toothpaste slurry prior to treatment of enamel specimens in order to demonstrate fluoride effects on demineralization [18, 19].

Conventional *in vitro* models of erosion use flat enamel samples, which are immersed in an acid challenge without being held together in close proximity [20]. Such models simulate erosion that takes place on exposed tooth surfaces, but are less good at modelling the

events that occur interproximally. Enamel is thinnest at the cervical margin [21], and non carious cervical lesions arising from the loss of tooth tissue are often observed in this location [22] resulting in the exposure of dentine. The model used in the current study recreates the interdental crevice environment, allowing a close approximation of the chemistry that occurs in this confined space to be achieved and erosion in this area to be investigated with better accuracy.

The additional protection of the calcium silicate and sodium phosphate salts and fluoride containing toothpaste versus the SMFP control toothpaste observed in both studies is most likely due to the deposition and retention of calcium silicate particles onto the enamel surfaces [14, 16]. The protective effects of a toothpaste formulation containing calcium silicate, sodium phosphate salts and fluoride versus control toothpaste formulations have been previously confirmed in a series of *in vitro* studies [9]. The deposition of calcium silicate particles onto enamel surfaces have been shown to reduce the subsequent impact of acid challenges by decreasing the intrinsic rate constant of calcium loss from the enamel surface by at least 39% when compared to a non-treated control enamel surface [14]. The protective effects of calcium silicate may be due to a number of possible mechanisms. For example, calcium silicate can release calcium ions into the surrounding oral fluids under acidic conditions and by raising the local calcium concentration will increase the degree of saturation with respect to enamel hydroxyapatite (HAP) and inhibit dissolution [1, 16]. Calcium silicate may act as a chemical barrier where its pH buffering capabilities will inhibit localized pH drop and subsequent acid damage to enamel, or it may act as a physical barrier. Furthermore, calcium silicate has the ability to nucleate HAP which can shift the equilibrium towards remineralization and result in overall reduction in enamel mineral loss [14, 16]. In addition, the nucleated HAP may act as a sacrificial material to the enamel during acid exposure.

In the second study, with increased frequency of acid exposure, it was shown that the combination of toothpaste and dual-phase gel protected the enamel surface from significant acid softening compared to baseline after 3 days, whereas all other treatments showed a significant reduction in hardness after 3 days at the higher frequency acid exposure. The enhanced protection provided in the second study by the addition of the dual-phase gel may be due to a number of factors including, the effect of greater available fluoride levels provided from sodium fluoride aiding in the prevention of further demineralization [23] and the longer contact time provided by the dual-phase gel product to the enamel surface leading to more effective delivery and retention of calcium silicate particles to the enamel surfaces. Previous work by Parker *et al* [14] and Sun *et al* [16] have shown that calcium silicate is deposited onto sound and acid etched enamel and transforms into HAP but it appears that calcium silicate deposits to a larger extent onto etched enamel than sound enamel. This may explain the greater protection from the combined toothpaste

and dual-phase gel in the second study as it promotes the repair of demineralized enamel to a greater extent than the toothpaste alone [8, 9].

Previous work has shown that when using human and bovine enamel specimens the amount of surface softening increases as the acid exposure time increases [24]. Generally in this study, doubling the frequency of acid exposure increased the amount of softening approximately three times which initially may seem high but the amount of continuous time in the artificial saliva was also reduced thus highlighting the possible importance of remineralization for reducing the rate of early erosion [25]. Increasing the acid frequency had no effect on the specimens during the time that they were treated with the combined calcium silicate and sodium phosphate toothpaste and dual-phase gel. This indicates that this combination of products have potential to protect enamel from frequent acid exposures more than the toothpaste groups alone.

The SEM images acquired at each time point for both studies reflects the results discussed above. It is clear that the amount of surface change is greater following an increase in acid frequency.

## **5.0 Conclusions**

The results from this work have demonstrated how an in vitro interproximal model can be successfully used to determine differences in the erosion protection of various toothpastes and treatments as well as determining how they perform when the frequency of acid exposure is increased. Whilst this is a laboratory model it has been carefully designed to mimic the natural proximity of the tooth position and hence gives a more realistic dynamic flow of saliva and oral care products across the enamel. This model provides better evidence for efficacy of toothpastes than standard in vitro models.

## **Acknowledgements**

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## **References**

- [1] West NX, Joiner A. Enamel mineral loss. *J Dent* 2014; 42:S2-11. DOI:10.1016/S0300-5712(14)50002-4
- [2] West NX, Hughes JA, Addy M. The effect of pH on the erosion of dentine and enamel by dietary acids in vitro. *J Oral Rehabil* 2001; 28:860-4. DOI:10.1111/j.1365-2842.2001.00778.x

- [3] Young A, Tenuta LMA. Initial Erosion Models. *Caries Res* 2011; 45:33-42. DOI:10.1159/000325943
- [4] Tawakoli PN, Sauer B, Becker K, Buchalla W, Attin T. Interproximal biofilm removal by intervallic use of a sonic toothbrush compared to an oral irrigation system. *BMC Oral Health* 2015; 15:91. DOI:10.1186/s12903-015-0079-6.
- [5] Rmaile A, Carugo D, Capretto L, Aspiras M, De Jager M, Ward, M, Stoodley P. Removal of interproximal dental biofilms by high-velocity water microdrops. *J Dent Res* 2014; 93:68-73. DOI:10.1177/0022034513510945
- [6] Sarig R, Vardimon AD, Sussan C, Benny L, Sarne O, HersHKovitz I, Shpack N. Pattern of maxillary and mandibular proximal enamel thickness at the contact area of the permanent dentition from first molar to first molar. *Am J Orthod Dentofacial Orthop* 2015; 147:435-44. DOI:10.1016/j.ajodo.2014.11.026
- [7] West NX, Lussi A, Seong J, Hellwig E. Dentin hypersensitivity: pain mechanisms and aetiology of exposed cervical dentin. *Clin Oral Investig* 2013; 17:S9-S19. DOI:10.1007/s00784-012-0887-x
- [8] Jones SB, Davies M, Chapman N, Wilson R, Hornby K, Joiner A, West NX. Introduction of an interproximal mineralisation model to measure remineralisation caused by novel formulations containing calcium silicate, sodium phosphate salts and fluoride. *J Dent* 2014; 42:S46-S52. DOI:10.1016/S0300-5712(14)50007-3
- [9] Hornby K, Ricketts SR, Philpotts CJ, Joiner A, Schemehorn B, Wilson R. Enhanced enamel benefits from a novel toothpaste and dual phase gel containing calcium silicate and sodium phosphate salts. *J Dent* 2014; 42:S39-S45. DOI:10.1016/S0300-5712(14)50006-1
- [10] Lussi A, Carvalho TS. The future of fluorides and other protective agents in erosion prevention. *Caries Res* 2015; 49:18-29. DOI:10.1159/000380886
- [11] Jones SB, Rees GD, Shellis RP, Barbour ME. The effect of monoalkyl phosphates and fluoride on dissolution of hydroxyapatite, and interactions with saliva. *Caries Res* 2013; 47:355-63. DOI:10.1159/000348594
- [12] Schlueter N, Klimek J, Ganss C. Randomised in situ study on the efficacy of a tin/chitosan toothpaste on erosive-abrasive enamel loss. *Caries Res* 2013; 47:574-81. DOI:10.1159/000351654
- [13] Lussi A, Megert B, Eggenberger D, Jaeggi T. Impact of different toothpastes on the prevention of erosion. *Caries Res* 2008; 42:62-7. DOI:10.1159/000112517
- [14] Parker AS, Patel AN, Botros RA, Snowden ME, McKelvey K, Unwin PR, Ashcroft AT, Carvell M, Joiner A, Peruffo M. Measurement of the efficacy of calcium silicate for the

protection and repair of dental enamel. J Dent 2014; 42:S21-9. DOI:10.1016/S0300-5712(14)50004-8

[15] Joiner A, Schäfer F, Naeeni MM, Gupta AK, Zero DT. Remineralisation effect of a dual-phase calcium silicate/phosphate gel combined with calcium silicate/phosphate toothpaste on acid-challenged enamel in situ. J Dent 2014; 42:S53-9. DOI:10.1016/S0300-5712(14)50008-5

[16] Sun Y, Li X, Deng Y, Sun JN, Tao DY, Chen H, Hu Q, Liu R, Liu W, Feng X, Wang J, Carvell M, Joiner A. Mode of action studies on the formation of enamel minerals from a novel toothpaste containing calcium silicate and sodium phosphate salts. J Dent 2014; 42:S30-8. DOI:10.1016/S0300-5712(14)50005-X

[17] Tenuta LMA, Cury JA. Laboratory and human studies to estimate anticaries efficacy of fluoride toothpastes. Monogr Oral Sci 2013; 23:108-24. DOI:10.1159/000350479

[18] Lynch RJM, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel: role of fluoride toothpastes. Int Dent J 2004; 54:304-9. DOI:10.1111/j.1875-595X.2004.tb00003.x

[19] Hornby K, Evans M, Long M, Joiner A, Laucello M, Salvaderi A. Enamel benefits of a new hydroxyapatite containing fluoride toothpaste. Int Dent J 2009; 59:325-31. DOI:10.1002/idj.2009.59.6s1.325

[20] Oliveira GC, Tereza GPG, Boteon AP, Ferrairo BM, Gonçalves PSP, Silva TCD, Honório HM, Rios D. Susceptibility of bovine dental enamel with initial erosion lesion to new erosive challenges. PLoS One; 2017;12:e0182347. DOI: 10.1371/journal.pone.0182347

[21] Gaspersic D. Micromorphometric analysis of cervical enamel structure of human upper third molars. Arch Oral Biol 1995;40:453-7. DOI: 10.1016/0003-9969(94)00180-J

[22] Hunter ML, Addy M, Pickles MJ, Joiner A. The role of toothpastes and toothbrushes in the aetiology of tooth wear. Int Dent J 2002;52:399-405. DOI: 10.1111/j.1875-595X.2002.tb00729.x

[23] Magalhaes AC, Wiegand A, Rios D, Buzalaf AMR, Lussi A. Fluoride in dental erosion. Monogr Oral Sci 2011; 22:158-70. DOI:10.1159/000325167

[24] White AJ, Yorath C, ten Hengel V, Leary SD, Huysmans MC, Barbour ME. Human and bovine enamel erosion under 'single-drink' conditions. Eur J Oral Sci 2010; 118:604-9. DOI:10.1111/j.1600-0722.2010.00779.x

[25] Amaechi BT, Higham SM. In vitro remineralisation of eroded enamel lesions by saliva. J Dent 2001; 29:371-6. DOI:10.1016/S0300-5712(01)00026-4

## Figures

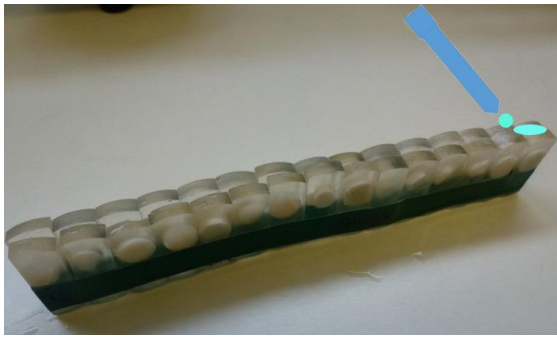


Figure 1. Image of the enamel specimens in their interproximal position with the paste being added to the row of interproximally positioned specimens prior to rinsing and placing into artificial saliva.

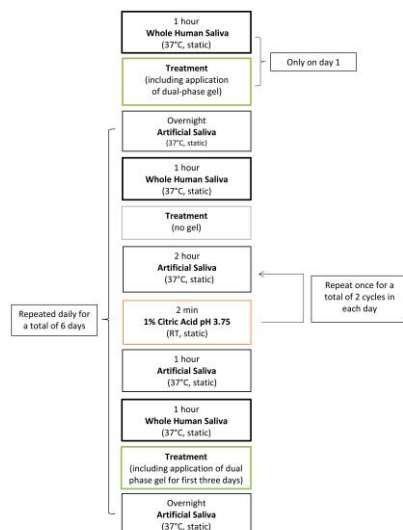


Figure 2. Summary of the treatment regime. RT: room temperature.

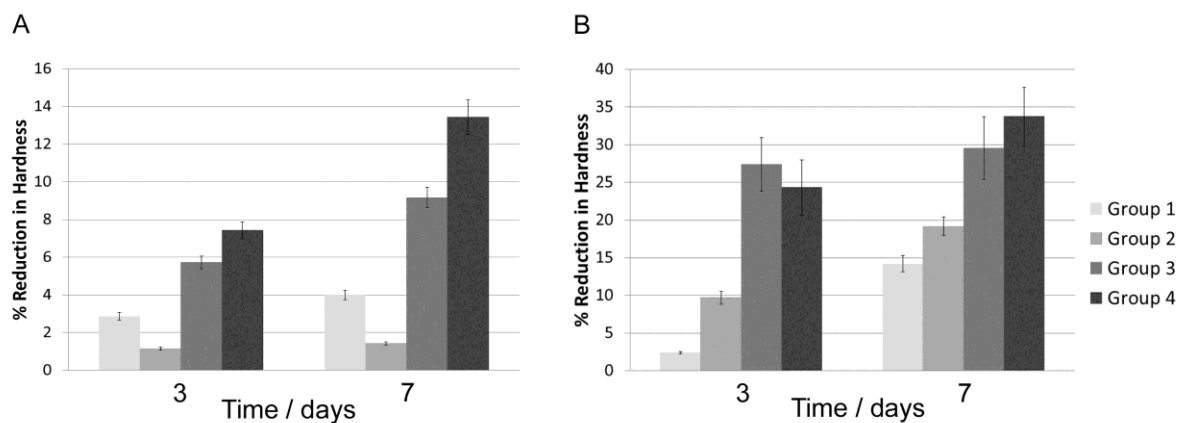


Figure 3. Graphs showing the average % reduction in microhardness from baseline for each group after 3 and 7 days of acid/treatment cycling. (A) Study 1, (B) Study 2.

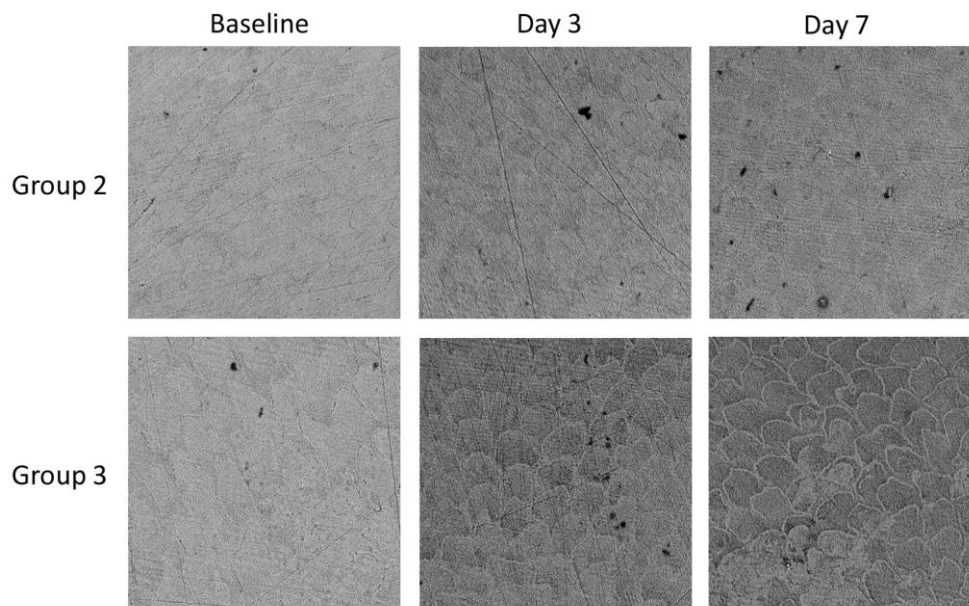


Figure 4. Representative SEM images of the enamel surface at baseline and following treatment for three and seven days.

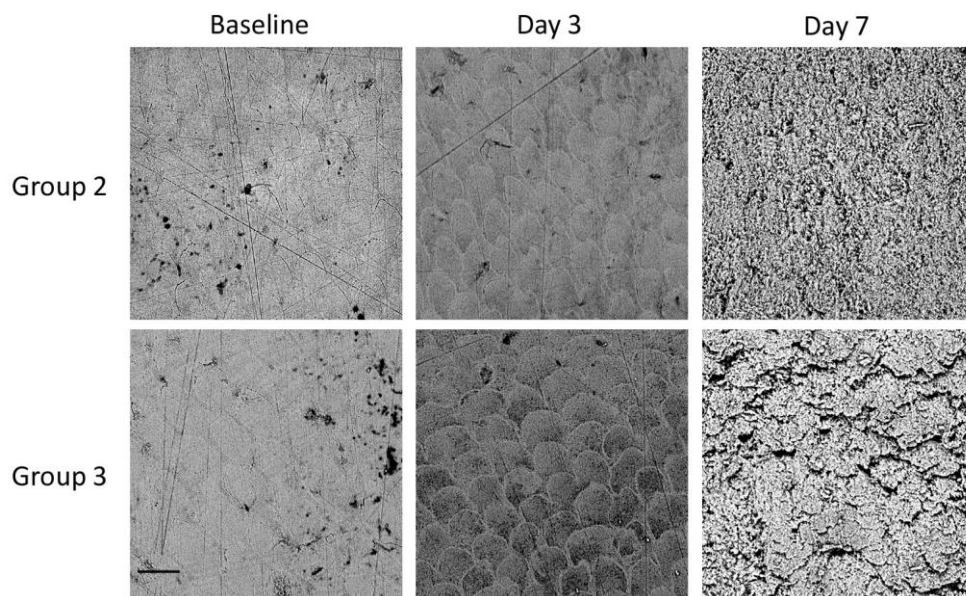


Figure 5. Representative SEM images of the enamel surface at baseline and following treatment for three and seven days. Scale bar = 5 $\mu$ m

Table 1. Treatment groups

<b>Specimen Group</b>	<b>Treatment</b>	<b>Active Ingredient</b>
<b>1</b>	Toothpaste containing calcium silicate and sodium phosphate  + 3 days of dual phase gel (gel applied following only the evening treatment on the first 3 consecutive days)	calcium silicate and sodium phosphate salts with 1450 ppm F as SMFP  calcium silicate and sodium phosphate salts with 1450 ppm F as SMFP and NaF
<b>2</b>	Toothpaste containing calcium silicate and sodium phosphate	calcium silicate and sodium phosphate salts with 1450 ppm F as SMFP
<b>3</b>	Fluoride containing toothpaste	1450 ppm F as SMFP
<b>4</b>	Non-fluoride toothpaste	-



Table 2. Mean hardness (VHN) with standard deviations in parentheses. Statistically significant differences are denoted by lowercase letters (between groups at each time point) and uppercase letters (within groups) ( $p < 0.05$ ).

	<b>VHN Baseline</b>	<b>VHN Day 3</b>	<b>VHN Day 7</b>
<b>Group 1</b>	350 (22) <sup>a, A</sup>	340 (25) <sup>a,b, A</sup>	336 (21) <sup>a, A</sup>
<b>Group 2</b>	350 (25) <sup>a, A</sup>	346 (24) <sup>a, A</sup>	344 (21) <sup>a, A</sup>
<b>Group 3</b>	349 (23) <sup>a, A</sup>	329 (19) <sup>a,b, B</sup>	317 (19) <sup>b, B</sup>
<b>Group 4</b>	350 (25) <sup>a, A</sup>	324 (20) <sup>b, B</sup>	303 (21) <sup>b, C</sup>

Table 3. Mean hardness (VHN). Standard deviations are in parentheses. Statistically significant differences are denoted by lowercase letters (between groups at each time point) and uppercase letters (within groups) ( $p < 0.05$ ).

	<b>VHN Baseline</b>	<b>VHN Day 3</b>	<b>VHN Day 7</b>
<b>Group 1</b>	356 (23) <sup>a, A</sup>	348 (24) <sup>a, A</sup>	305 (23) <sup>a, B</sup>
<b>Group 2</b>	358 (19) <sup>a, A</sup>	323 (28) <sup>a, B</sup>	289 (19) <sup>a, C</sup>
<b>Group 3</b>	360 (21) <sup>a, A</sup>	261 (34) <sup>b, B</sup>	253 (36) <sup>b, B</sup>
<b>Group 4</b>	361 (19) <sup>a, A</sup>	273 (41) <sup>b, B</sup>	239 (28) <sup>b, C</sup>

Table 4. Mean difference in surface softening from baseline values following each treatment period for specimens exposed to low (Study 1) and high (Study 2) frequency acid exposure. Standard deviations are in parentheses.

	<b>Day 3</b>		<b>Day 7</b>	
	<b>Study 1</b>	<b>Study 2</b>	<b>Study 1</b>	<b>Study 2</b>
<b>Group 1</b>	10.25 (22.13)	8.90 (17.67)	14.44 (17.67)	50.95 (25.04)
<b>Group 2</b>	4.12 (18.63)	34.66 (19.56)	5.42 (19.56)	68.50 (24.20)
<b>Group 3</b>	20.23 (23.19)	98.54 (40.93)	32.47 (40.93)	106.33 (43.17)
<b>Group 4</b>	25.70 (17.00)	87.67 (41.85)	46.92 (41.85)	121.68 (34.17)